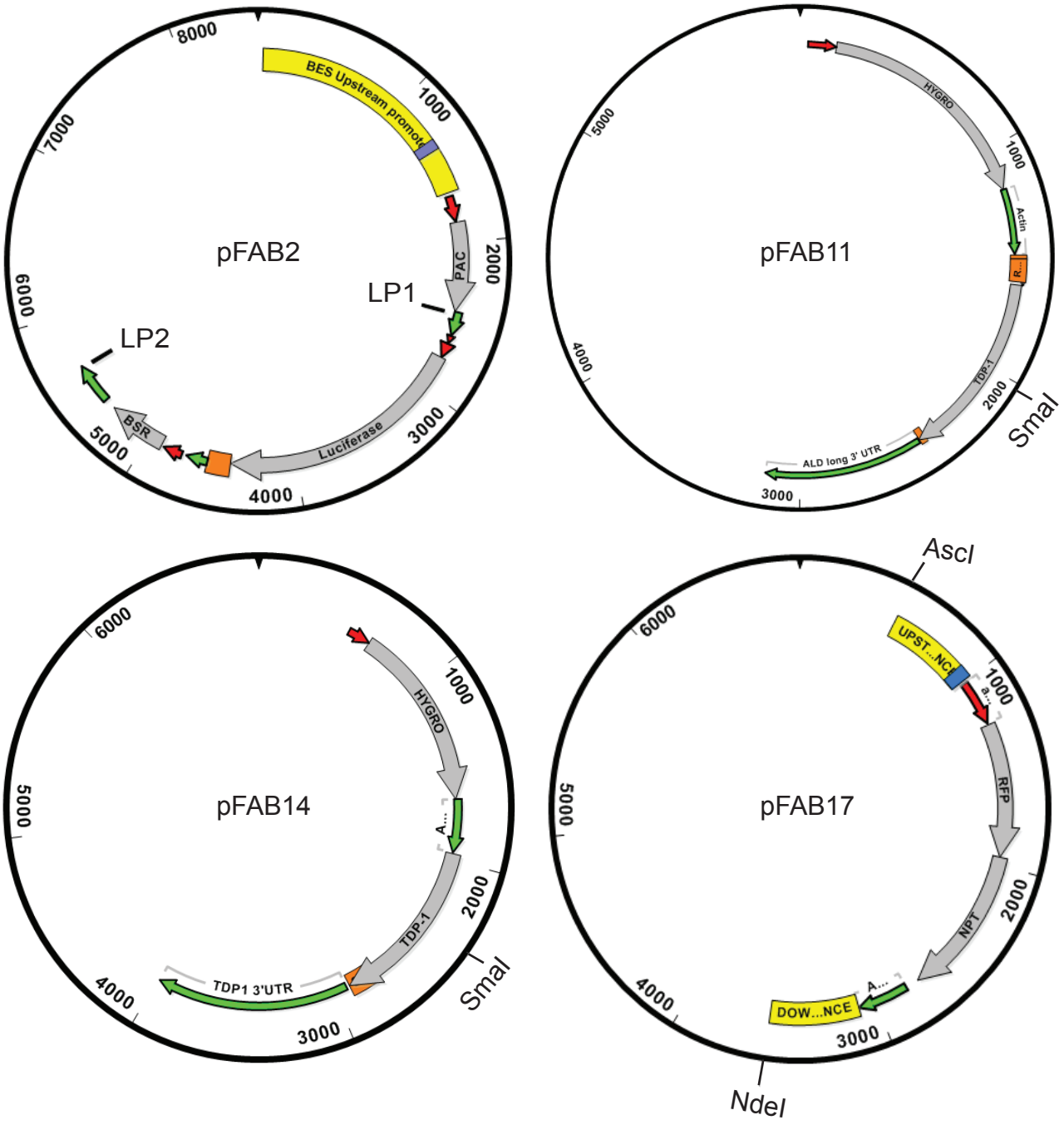
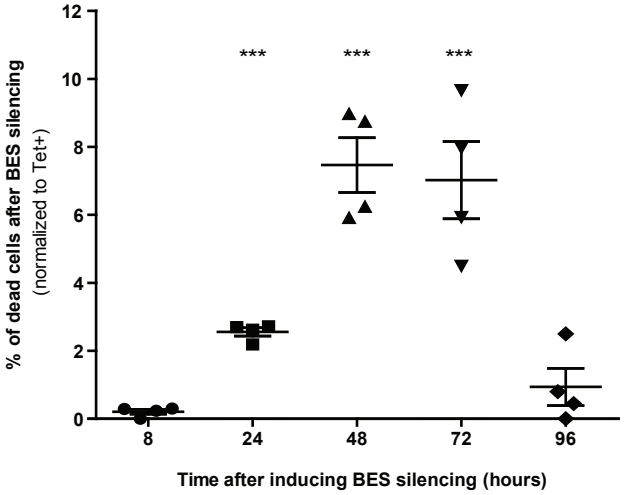


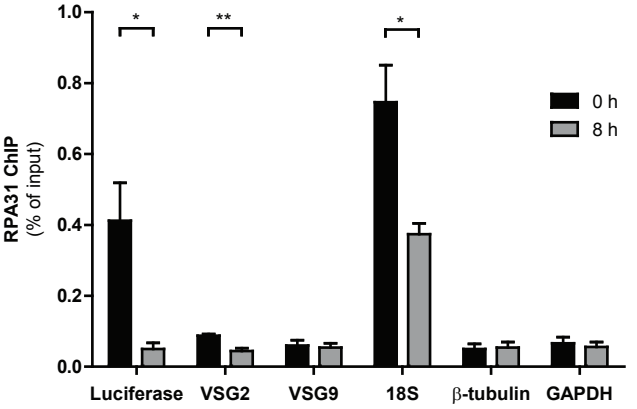
Supplementary Figure S1. Maps of constructs used in this work.

Constructs are described in Materials and Methods. Gene ORFs are represented in gray, 5'UTRs in red, 3'UTRs in green, upstream and downstream BES promoter sequences in yellow and BES promoter in blue. LP1 and LP2 represent the primers with long tails designed to generate GLB1 with the sequence listed in Supplementary Table 1. Restriction sites used prior to transfection are indicated.



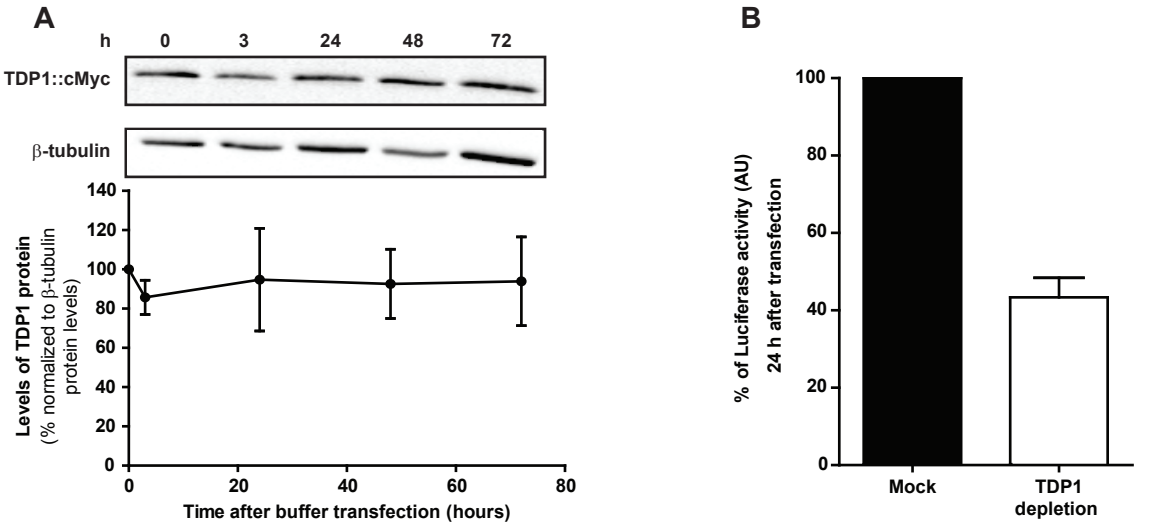
Supplementary Figure S2. Cell death during BES silencing assay.

BES silencing was induced and cells were collected 8, 24, 48, 72 and 96 h later. Induced cells were incubated with 5 μg/ml of propidium iodide (PI) prior to FACS analysis. Dead cells or in late apoptosis are stained by propidium iodide. The number of PI positive cells in 'Tet-' culture was normalized to the number of PI positive cells in 'Tet+' culture. Four independent experiments were analyzed. Statistical significance was determined by a t-test against a hypothetical mean value of 0, corresponding to no cell death. ***: $p < 0.001$.



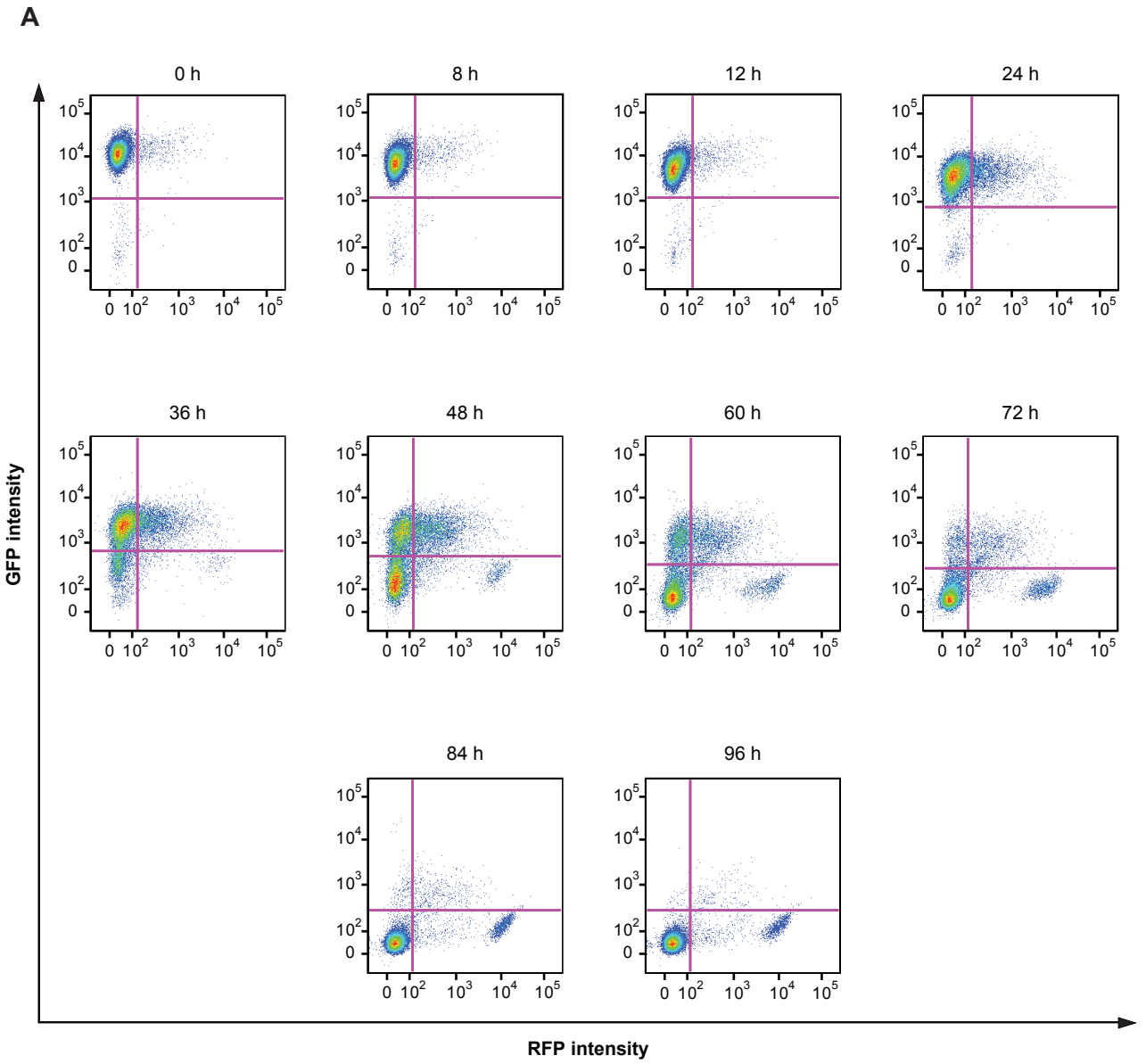
Supplementary Figure S3. RNA Pol I is absent from active BES 8 h after inducing BES silencing.

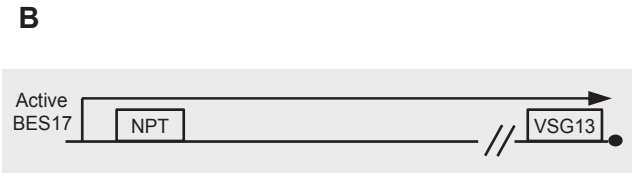
RNA Pol I occupancy was determined by RPA31 subunit ChIP at 0 and 8 h after tetracycline removal. Immunoprecipitated DNA was compared to the total input material. Five independent experiments were analyzed. Statistical significance was determined by a paired t-test against time-point 0 h. *:$p < 0.05$; **: $p < 0.01$.

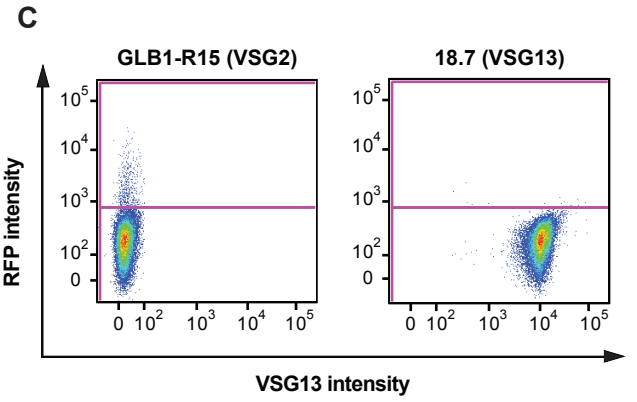


Supplementary Figure S4. TDP1 facilitates BES transcription.

(A) Western blotting analysis of TDP1 protein after 3, 24, 48 and 72 h of transfection with buffer (mock) in GLB1-TDP1::3xcMyc. Time-point 0 h indicates cells which were not transfected. Each lane corresponds to lysates from 2×10^6 cells. Quantification of TDP1 signal is indicated in the lower panel. TDP1 protein levels were normalized for β-tubulin protein levels and to not transfected cells. Four independent experiments were analyzed. **(B)** Luciferase activity was measured 24 h after TDP1 depletion and compared to the mock control in GLB1-TDP1::3xcMyc. Five independent experiments were analyzed.







Supplementary Figure S5. GFP and RFP gene expression during BES silencing.

(A) Representative density plots of GFP and RFP intensities of GLB1-R15 after induction of BES silencing. Cells were analyzed by FACS from 0 to 96 h. X-axis represents RFP intensity while Y-axis represents GFP intensity. As indicated in **Figure 6B**, *GFP* is present in originally active BES1 and *RFP* is present in the originally silent BES15. **(B)** The cell-line 18.7, which expresses VSG13. *NPT*, Neomycin Phosphotransferase. **(C)** Representative density plots of RFP and VSG13 intensities of GLB1-R15 (expresses VSG2, **left panel**) and 18.7 (expresses VSG13, **right panel**). X-axis represents VSG13 intensity while Y-axis represents RFP intensity. Top gate represents RFP positive cells and bottom gate represents RFP negative cells. This gating was applied in **Figure 6E**, **right panel**.